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Characterization of Bis-8-hydroxyquinoline-Armed Diazatrithia-16-crown-5 and Diazadibenzo-18-crown-6 Ligands as Fluorescent Chemosensors for Zinc

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Key words: fluorescent chemosensor, zinc, complexation, ion selectivity

Abstract

Bis-8-hydroxyquinoline-armed diazatrithia-16-crown-5 (**1**) and diazadibenzo-18-crown-6 (**2a**) ligands selectively respond to Zn^{2+} over other tested metal ions, including Cd^{2+} , via large increases in fluorescence, while other side-armed diazadibenzo-18-crown-6 ligands (**2b-f**) and bis(dansylamidoethyl)-armed 1,8-dimethyltetraza-14-crown-4 ligand (**3**) do not respond to tested metal ions via an increase in fluorescence. Ligand **1** forms a 1:1 complex (ML) and a 1:2 complex (M_2L) with Zn^{2+} , and these have emission maxima at different wavelengths (540 and 500 nm, respectively). Due to the large fluorescent enhancement factor of ligand **1** for Zn^{2+} , ligand **1** is well suited for use as a fluorescent chemosensor for Zn^{2+} .

Introduction

The development of selective and sensitive chemosensors for quantitative analysis of Zn^{2+} has become extremely important for environmental and biological applications [1, 2]. While remarkable progress has been made in development of chemosensors for other biologically important divalent metal ions such as Ca^{2+} (e.g., Fura-2, Quin-2) [3], there are few Zn^{2+} -selective analytical reagents available. Reported Zn^{2+} chelating fluorophores use a variety of strategies for binding and responding to their target metal ion [4]. Polyamines and macrocyclic ligands have been employed for ion binding, and these groups have been tethered to fluorophores including derivatives of fluorescein [5,6], xanthene [7], dansyl [8], anthracene [9] and quinoline [10,11]. Because Cd^{2+} is often found with Zn^{2+} in the environment [12] and can form fluorescent complexes with chelating fluorophores [13], a potentially important property of chemosensors for Zn^{2+} is their selectivity for Zn^{2+} over Cd^{2+} . Ion selectivity can originate through selective association with the target analyte and/or selective response to the target analyte.

We have prepared series of macrocyclic ligands appended with metal-ion chelating fluorophores designed to act as chemosensors for metal ions, including Zn^{2+} , and one of our aims is the development of fluorescent chemosensors with high selectivity for Zn^{2+} . The Zn^{2+} vs. Cd^{2+} selectivity of reported Zn^{2+} chemosensors has

generally not been described. We recently reported the synthesis and preliminary photophysical studies of bis-8-hydroxyquinoline-armed diazatrithia-16-crown-5 (**1**) (Figure 1), which exhibited increased fluorescence in the presence of Zn^{2+} [11]. We have also described the preparation of series of diazadibenzo-18-crown-6 ligands containing two 8-hydroxyquinoline sidearms or other two sidearms (**2a-f**), and 1,8-dimethyltetraza-14-crown-4 ligand containing two dansylamidoethyl sidearms (**3**) (Figure 1)[14, 15]. To determine if these potential chemosensors display high selectivity for Zn^{2+} over Cd^{2+} , the fluorescence responses of these compounds were carefully compared in the presence of Zn^{2+} , Cd^{2+} and other transition metal ions.

Experimental

Synthesis

The preparation of the compounds **1**, **2a-f**, and **3** were reported in Ref. 11, 14 and 15.

Measurements

UV-vis spectral measurements were carried out using a previously described procedure [16]. The ligand stock solutions were prepared by dissolution of a weighed amount of ligand in methanol (HPLC grade, Fisher Scientific or Spectrophotometric grade, Malinckrodt). Titrations of the ligand ($C_L = 1 \times 10^{-5} \text{ M}$) by metal ion solutions were performed directly in a spectrophotometric cell of 1 cm path length. The resulting spectra were recorded from 190 to 1100 nm at room temperature with an HP 8453 spectrophotometer after each addition of metal salt. The ionic strength was kept constant at 0.01 M by addition of sodium acetate (certified, Fisher Scientific). The following metal salts were used: $\text{Cu}(\text{NO}_3)_2$, and $\text{Zn}(\text{NO}_3)_2$ (certified A.C.S., Fisher Scientific), $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (AR, Mallinckrodt), and $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$. The concentrations of stock solutions of metal salts were determined by complexometric titration with EDTA in the presence of the appropriate indicator [17].

Fluorescence spectra were measured using $\text{ex} = 390$ (for ligands **1** and **3**) or 253 (for ligands **2a-f**) nm at wavelengths between 400 and 700 nm with a Horiba FluoroMax-3 fluorimeter. Fluorescence intensities were measured in methanol containing 0.01 M NaOAc. The titrations were performed with titrant (metal ions; 1-100 μM) and titrate (ligand; 10 μM). The metal ion sources were identical to those used to perform the UV-vis studies.

Results and discussion

Absorption studies

We examined the change of the absorption spectra in adding Zn^{2+} and Cd^{2+} to the solution of ligands **1**, **2a-f**, and **3**. Addition of these metal ions resulted in no observable changes in the absorption spectra of ligands **2b-f** and **3**. However, addition of Zn^{2+} or Cd^{2+} to **1** and **2a** resulted in a blue shift of the quinoline absorption (for example, Figure 2 shows the absorption spectra of **2a** in the absence and presence of Zn^{2+}). The

absorption spectra of **1** in the presence of Zn^{2+} suggested that both 1:1 (ligand:ion) and 1:2 complexes formed [11]. In the study of **1** with Zn^{2+} the first series of spectral lines passed through two isosbestic points at 226.5 and 253nm until $C_M/C_L \approx 1$, and a new isosbestic point at 261nm was observed at higher values of C_M/C_L . The second isosbestic point is consistent with 1:2 complex formation. This behavior was not observed in complex formation of **1** with Cd^{2+} , and **2a** with Zn^{2+} or Cd^{2+} ; only formation of 1:1 complexes were observed.

Fluorescence studies

The fluorescence spectra of **1** in the presence of several concentrations of $\text{Zn}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$ are shown in Figures 3a and 3b, respectively. As described [11], the Zn^{2+} complex of **1** gave a strong emission band, and bands from the 1:1 and 1:2 complexes have emission maxima at different wavelengths (540 and 500 nm, respectively) (Figure 3a). The Cd^{2+} complex of **1** exhibited weaker fluorescence intensity and formed only a 1:1 complexes.

Titration curves of **1** with Zn^{2+} , Cd^{2+} and Pb^{2+} at 550 nm and 475 nm are shown in Figure 4a and 4b. The titration curves at 550 nm indicate a sharp endpoint for **1** at a 1:1 ligand:ion ratio for Cd^{2+} and Pb^{2+} , and with Zn^{2+} show formation of the 1:2 complex. The 1:1 complex of **1** and Zn^{2+} fluoresces minimally at 475 nm. Likewise, the complexes of Cd^{2+} and Pb^{2+} with **1** are not fluorescent at 475 nm. Consequently, monitoring the titration at 475nm showed only formation of the 1:2 complex of **1** and Zn^{2+} without interference from Cd^{2+} and Pb^{2+} . The fluorescence enhancement factors at 475nm were 32, 2.4 and 1.4 for Zn^{2+} , Cd^{2+} and Pb^{2+} , respectively (Table 1).

We also examined the fluorescence response of **1** to mixtures of metal ions. Figure 5a and 5b illustrate the titration curves of **1** with Zn^{2+} - Cd^{2+} , Zn^{2+} - Pb^{2+} , Zn^{2+} - Cu^{2+} , and Zn^{2+} - Hg^{2+} (1:1 solutions of the metal ions) at 550nm and 475nm. The titrations monitored at 550nm (Figure 5a) showed that **1** formed a 1:1 complex with Zn^{2+} in the presence of these other metal ions, but that at high metal ion concentrations Cu^{2+} and Hg^{2+} out competed Zn^{2+} for the ligand yielding only non-fluorescent complexes with **1**. The titrations monitored at 475nm showed that the 1:2 complex of **1** and Zn^{2+} formed in the presence of Cd^{2+} and Pb^{2+} , but not in the presence of Cu^{2+} and Hg^{2+} . Fluorescence enhancement factors are shown Table 1.

To determine the potential of ligands **2a-f** to act as effective chemosensors, fluorescence titrations, similar to those described with **1**, were performed with these ligands. When Zn^{2+} and Cd^{2+} were added to ligands **2b-f** only very small changes in their fluorescence spectra were observed. In contrast, the fluorescence behavior of **2a** varied greatly in the presence of Zn^{2+} , and the **2a** - Zn^{2+} complex proved to be highly fluorescent (Figure 6). Only a slight increase in fluorescence of **2a** was observed in the presence of Cd^{2+} (fluorescence enhancement factors are given in Table 1).

Titration results of **2a** with Zn^{2+} , Zn^{2+} - Cd^{2+} , Zn^{2+} - Cu^{2+} , and Zn^{2+} - Hg^{2+} monitored at 530nm, respectively, are shown in Figure 7. These results suggest that ligand **2a** forms a 1:1 complex with Zn^{2+} , and that Cd^{2+} does not appear to greatly influence the response of **2a** to Zn^{2+} . However, as observed with **1**, Cu^{2+} and Hg^{2+} appear to out compete Zn^{2+} for the ligand, and at high metal ion concentrations non-fluorescent complexes form with these metal ions. Fluorescence enhancement factors are given in Table 1.

Dansyl groups have been used in the preparation of metal ion chemosensors with some success (for example, see Ref. 8). Consequently, we anticipated that ligand **3**

would prove useful as a chemosensor. Unlike hydroxyquinolines, which are only weakly fluorescent alone, dansyl groups are fluorescent without metal ion complexation. Therefore, metal ion complexation is monitored as modulation of fluorescence. However, addition of Zn^{2+} , Cd^{2+} and Pb^{2+} to **3** only resulted in modest decreases in fluorescence. Furthermore, the responses of **3** to Zn^{2+} and Cd^{2+} were nearly identical (Figure 8).

Conclusion

Bis-8-hydroxyquinoline-armed diazatrithia-16-crown-5 (**1**) and diazadibenzo-18-crown-6 (**2a**) ligands selectively respond to Zn^{2+} over other tested metal ions via a large increase in fluorescence. However, the other side-armed diazadibenzo-18-crown-6 ligands (**2b-f**) and bis(dansylamidoethyl)-armed 1,8-dimethyltetraza-14-crown-4 ligand (**3**) do not respond to tested metal ions via significant changes in fluorescence. These results suggest that 8-hydroxyquinoline sidearms are well suited for binding and responding to Zn^{2+} . Both ligands **1** and **2a** selectively bind Zn^{2+} over Cd^{2+} , and the fluorescence enhancement factor of **1** for Zn^{2+} is large. In addition, **1** forms both 1:1 and 1:2 complexes only with Zn^{2+} . These complexes fluoresce at different wavelengths allowing selective observation of each complex. However, the response of **1** to Zn^{2+} is modulated by Cd^{2+} . Ligand **2a** also responds to the presence of Zn^{2+} via a large increase in fluorescence, and the response of this ligand to Zn^{2+} is not significantly altered by Cd^{2+} . Both **1** and **2a** form non-fluorescent complexes with Cu^{2+} and Hg^{2+} ; consequently, for these ligands to be useful in determining Zn^{2+} concentrations, it will be important to ensure that the ligands are not used in limiting amounts. Due to their large changes in fluorescence in the presence of Zn^{2+} , and their ion selectivity, **1** and **2a** appear well suited for use as chemosensors for Zn^{2+} .

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Table 1. The enhancement factors (E.F.)^a of ligand **1** (at 475 nm) and ligand **2a** (at 530 nm).

	Zn ²⁺	Cd ²⁺	Pb ²⁺	Zn ²⁺ -Cd ²⁺	Zn ²⁺ -Pb ²⁺	Zn ²⁺ -Cu ²⁺	Zn ²⁺ -Hg ²⁺
1	32	2.4	1.4	11	32	4.0	2.2
2a	8.9	1.4	—	7.9	—	3.6	3.0

^aThe enhancement factor is the ratio of the fluorescence intensity of a complex divided by the fluorescence intensity of the uncomplexed ionophore.

Figure Captions

Figure 1. Structures of compounds **1**, **2a-f**, and **3**.

Figure 2. Spectral changes in the UV-vis absorption of **2a** ($C_L = 1 \times 10^{-5} M$) upon addition of $Zn(NO_3)_2$ in MeOH.: $0 < C_M/C_L < 10$)

Figure 3. a) Fluorescence spectra of the **1**- Zn^{2+} complex: $[1] = 10 \mu M$, $Zn^{2+} = 0.1$ - 10 equiv. b) Fluorescence spectra of the **1**- Cd^{2+} complex: $[1] = 10 \mu M$, $Cd^{2+} = 0.1$ - 10 equiv.

Figure 4. a) Fluorescence intensity (550 nm) of **1** ($1 \mu M$) titrated with Zn^{2+} , Cd^{2+} and Pb^{2+} (0.1 - $10 \mu M$) in MeOH containing $0.01 M$ NaOAc. b) Fluorescence intensity (475 nm) of **1** ($1 \mu M$) titrated with Zn^{2+} , Cd^{2+} and Pb^{2+} (0.1 - $10 \mu M$) in MeOH containing $0.01 M$ NaOAc.

Figure 5. a) Fluorescence intensity (550 nm) of **1** ($1 \mu M$) titrated with Zn^{2+} - Cd^{2+} , Zn^{2+} - Pb^{2+} , Zn^{2+} - Cu^{2+} , and Zn^{2+} - Hg^{2+} (0.1 - $10 \mu M$) in MeOH containing $0.01 M$ NaOAc. b) Fluorescence intensity (475 nm) of **1** ($1 \mu M$) titrated with Zn^{2+} - Cd^{2+} , Zn^{2+} - Pb^{2+} , Zn^{2+} - Cu^{2+} , and Zn^{2+} - Hg^{2+} (0.1 - $10 \mu M$) in MeOH containing $0.01 M$ NaOAc.

Figure 6. Fluorescence spectra of a titration of **2a** with Zn^{2+} : $[2a] = 10 \mu M$, $Zn^{2+} = 0.1$ - 10 equiv. The peak at 506 nm ($= 253 \text{ nm} \times 2$) is Rayleigh scattering.

Figure 7. Fluorescence intensity (530 nm) of **2a** ($1 \mu M$) titrated with Zn^{2+} , Zn^{2+} - Cu^{2+} , and Zn^{2+} - Hg^{2+} (0.1 - $10 \mu M$) in MeOH containing $0.01 M$ NaOAc.

Figure 8. Fluorescence intensity (520 nm) of **3** ($1 \mu M$) titrated with Zn^{2+} , Cd^{2+} and Pb^{2+} (0.1 - $10 \mu M$) in MeOH containing $0.01 M$ NaOAc.

Figure 1.

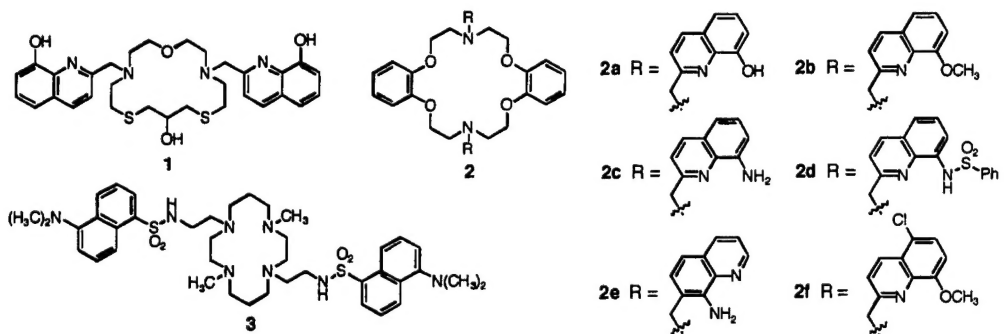


Figure 2.

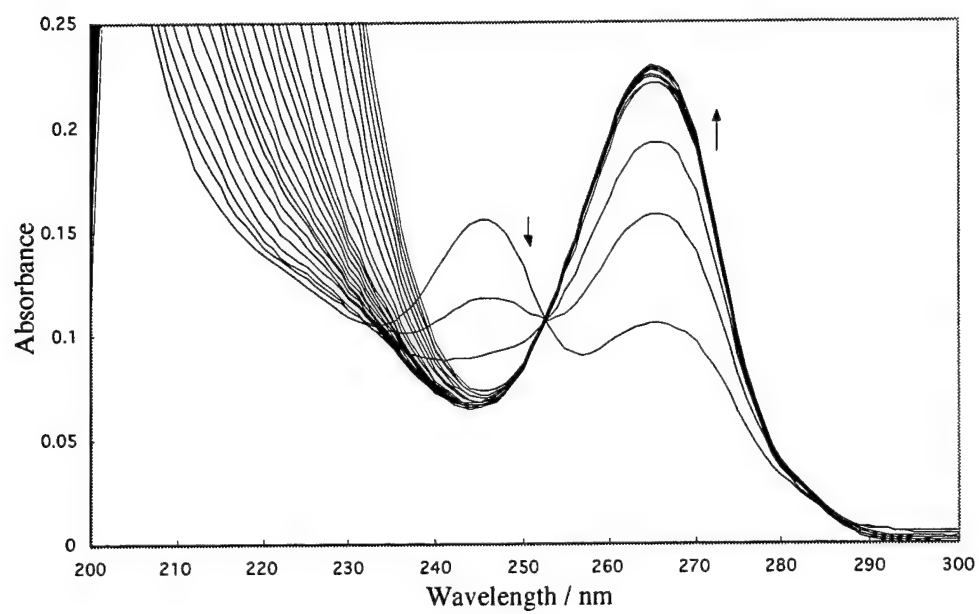


Figure 3.

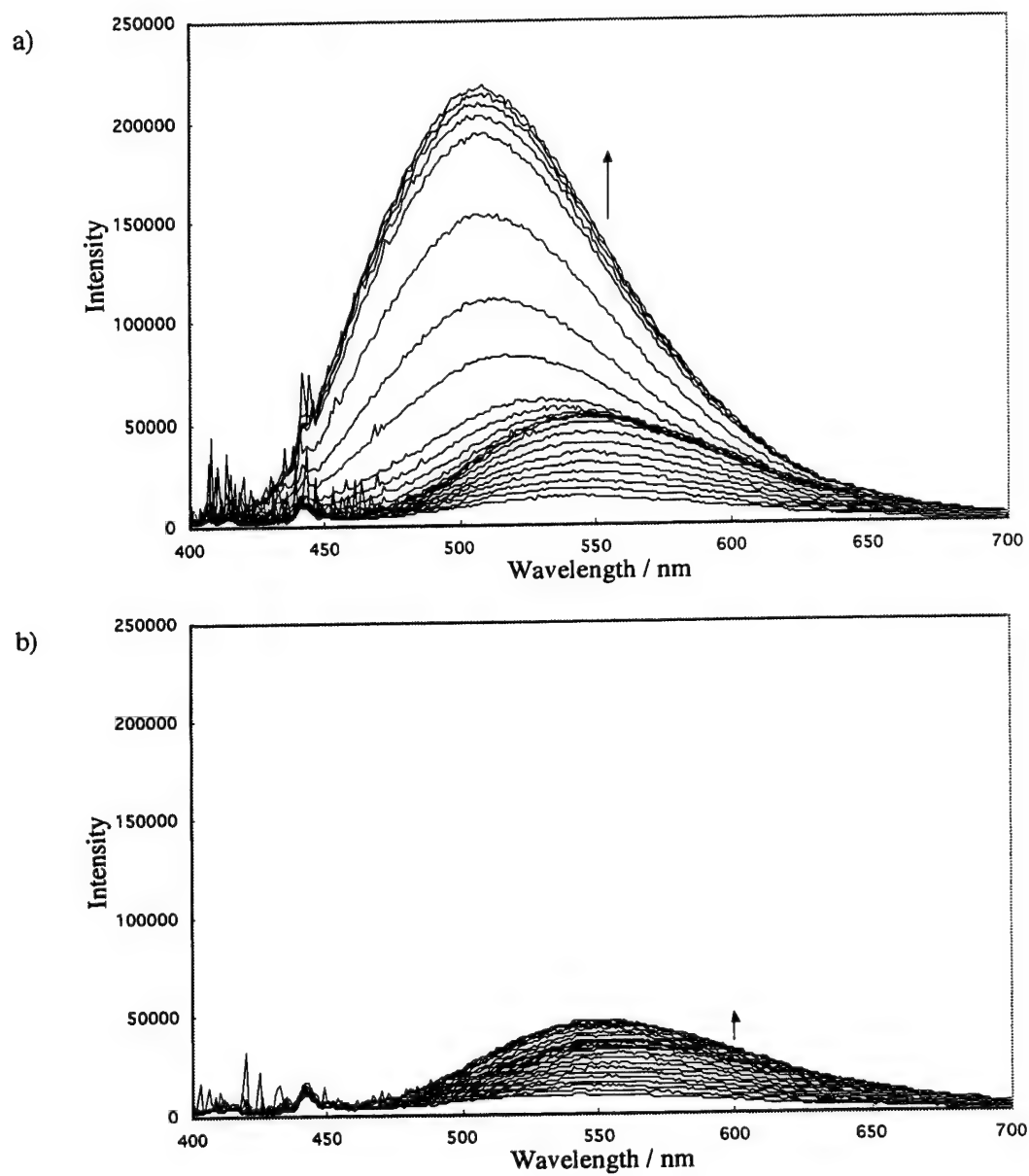


Figure 4.

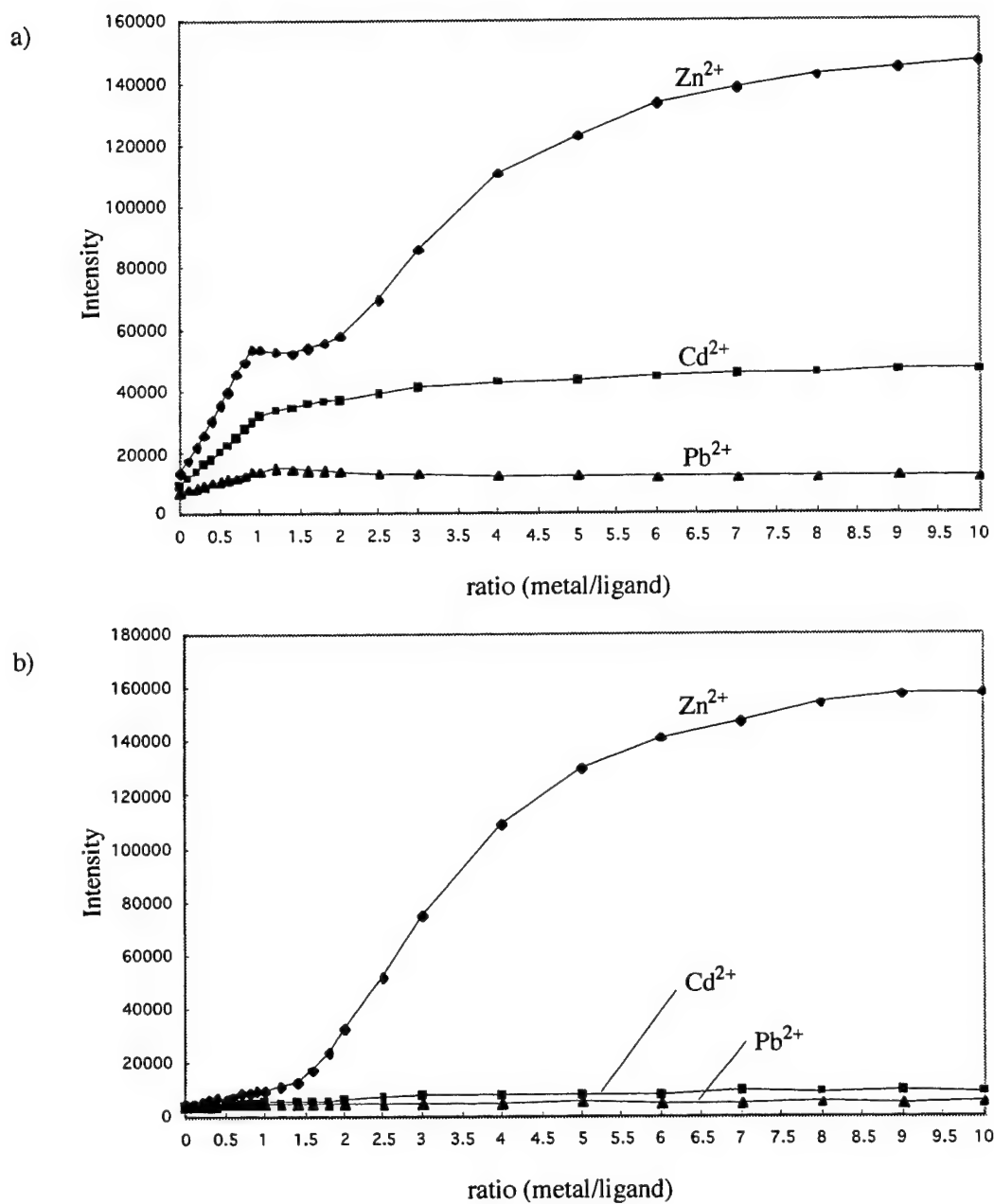


Figure 5.

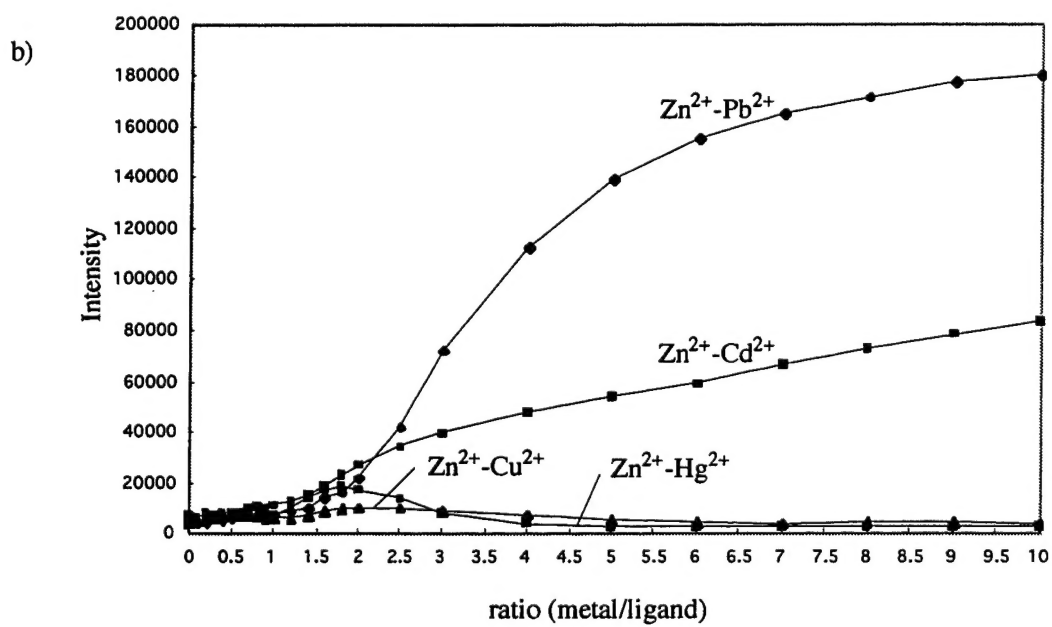
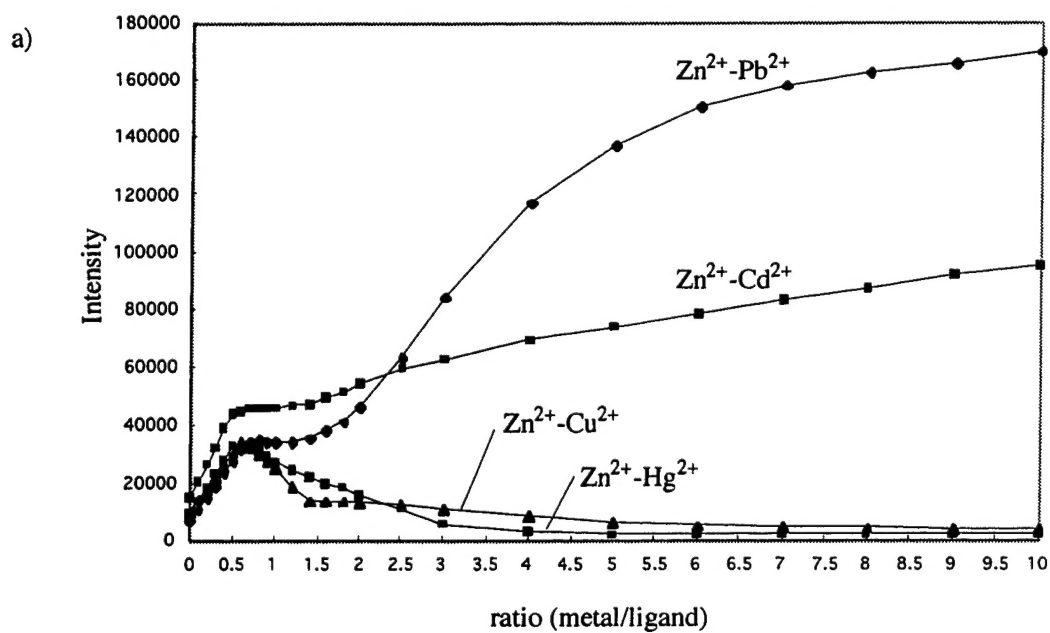


Figure 6.

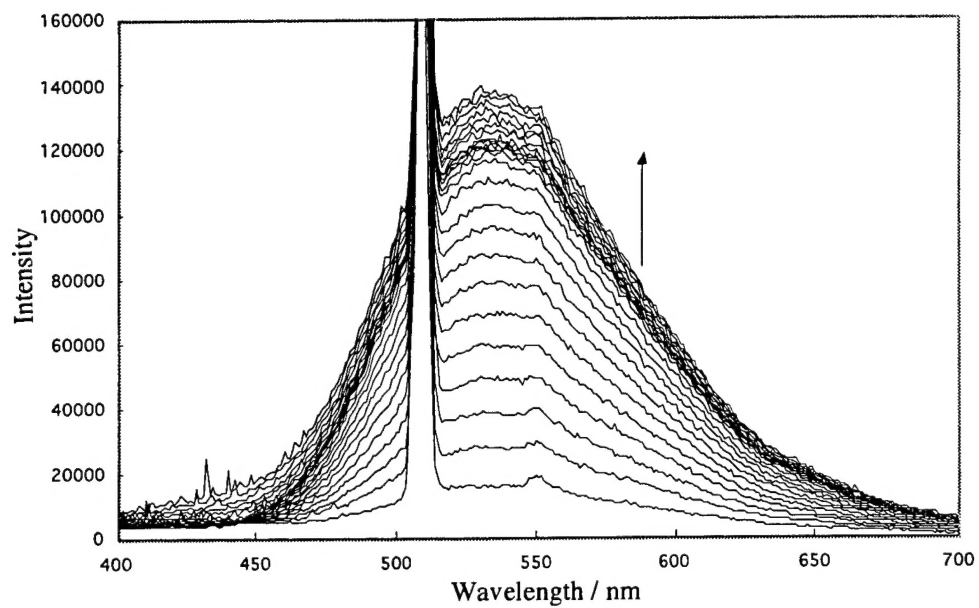


Figure 7.

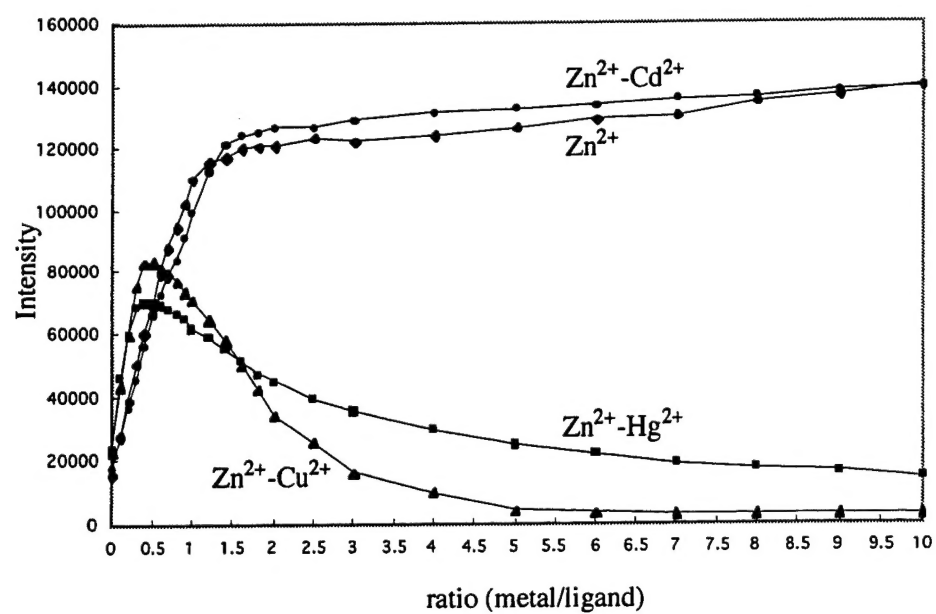


Figure 8.

